

# PATENT COOPERATION TREATY

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## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Rec'd PCT/PTO 04 OCT 2004  
16/509911

Applicant's or agent's file reference 618	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/DK 03/00220	International filing date (day/month/year) 04.04.2003	Priority date (day/month/year) 05.04.2002
International Patent Classification (IPC) or both national classification and IPC C07J41/00		
Applicant LEO PHARMA AS et al.		

<p>1. This International preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 8 sheets.</p>
<p>3. This report contains indications relating to the following items:</p> <p>I <input checked="" type="checkbox"/> Basis of the opinion</p> <p>II <input type="checkbox"/> Priority</p> <p>III <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p>IV <input type="checkbox"/> Lack of unity of invention</p> <p>V <input checked="" type="checkbox"/> Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p>VI <input type="checkbox"/> Certain documents cited</p> <p>VII <input type="checkbox"/> Certain defects in the international application</p> <p>VIII <input type="checkbox"/> Certain observations on the international application</p>

Date of submission of the demand  27.10.2003	Date of completion of this report  29.06.2004
Name and mailing address of the International preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer  Friebe, F  Telephone No. +49 89 2399-8552



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. **PCT/DK 03/00220**

**I. Basis of the report**

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17):*

**Description, Pages**

1-35 as originally filed

**Claims, Numbers**

1-36 filed with telefax on 14.04.2004

**Drawings, Sheets**

1/2-2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).  
☐ the language of publication of the international application (under Rule 48.3(b)).  
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.  
☐ filed together with the international application in computer readable form.  
☐ furnished subsequently to this Authority in written form.  
☐ furnished subsequently to this Authority in computer readable form.  
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☐ the claims, Nos.:  
☐ the drawings, sheets:

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5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 33-36

because:

☒ the said international application, or the said claims Nos. 33-36 - IA relate to the following subject matter which does not require an international preliminary examination (specify):

**see separate sheet**

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-24 - in part

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the Standard.

☐ the computer readable form has not been furnished or does not comply with the Standard.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes: Claims	1-36
	No: Claims	
Inventive step (IS)	Yes: Claims	
	No: Claims	1-36
Industrial applicability (IA)	Yes: Claims	1-32
	No: Claims	

2. Citations and explanations

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see separate sheet

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**point III:**

The subject-matter of Claims 1 to 24 has been only searched in part; the search is restricted to those parts which relate to compounds according to Claims 25 and 26 (see the comment adjacent to the Intern.Search Report).

Claims 33-36 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

**point V:**

The present application claims steroid derivatives containing up to 3 polyamine radicals; said compounds exhibit an antimicrobial activity.

Relevant prior art are the following documents:

- D1: **PINHAS H.et al.:** '6-amino derivatives of stigmastanol and cholestanol.' JOURNAL OF MEDICINAL CHEMISTRY. 1971, vol. 14, no. 11, November 1971 (1971-11), pages 1048-1049; see the compounds 5-7 and 10-12
- D2: **US-A-3 013 008 (COUNSELL RAYMOND E)** 12 December 1961 (1961-12-12); see the compounds of Claims 4,5 and 8-10
- D3: **BELLINI, A. et al.:** 'Antimicrobial activity of cholane compounds' EUR. J. MED. CHEM. vol. 18, no. 2, 1983, pages 185-190; see the compounds V, XII, XIX, XXVI

To delimit the presently scope of claims from these prior art compounds the Applicant has added a disclaimers at the end of Claim 1; Novelty is acknowledged (Art.33(2) PCT). For the sake of completeness the Applicant is already now informed that the admissibility of the disclaimer with regard to the 3rd reference will be decided in the reg.phase before the EPO (→no accidental disclosure).

As concerns the pharm.activity neither D1 nor D2 refers to an antibiotic activity. However, D3 explicitly underlines the antimicrobial activity of such compounds; this reference is therefore relevant under Art.33(3) PCT.

The same applies to the document

- D4: **WO 00 09137 A (RAO MEENA ;KINNEY WILLIAM (US); MAGAININ PHARMA (US); NOECKER LINC)** 24 February 2000 (2000-02-24)

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which is also highly relevant under Art.33(3) PCT.

The line of argumentation presented by the Applicant in order to overcome the obviousness objections is not persuasive; the Art.33(3) objection is maintained.

As concerns **D3** the following is to be noted:

The argument based on graphs 1 to 4 saying that the person skilled in the art would not take D3 into consideration in order to solve the problem addressed in the instant case is misleading. On the contrary, the cholane derivatives with the branched polyamine chain show indeed a high activity and therefore clearly constitute an incentive for the art skilled person to further elaborate this concept.

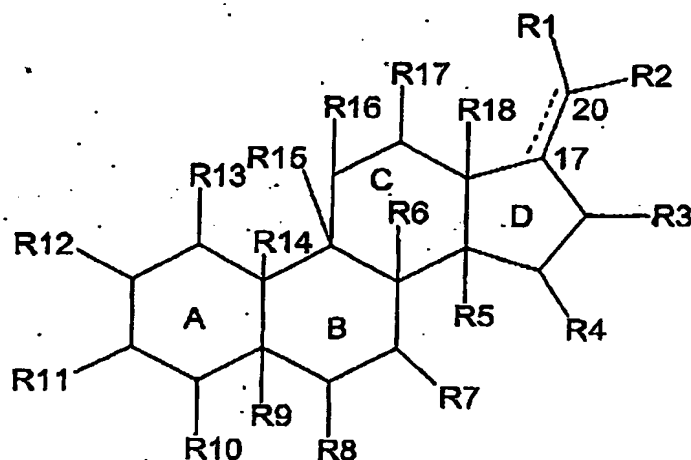
As concerns **D4** the Applicant in particular relies on the '*branched amine feature*' which according to his point of view is essential to the instantly claimed subject-matter. This however is not corroborated by the language of the claims presently on file. The proviso on page 1 of Claim 1 makes compulsory (as a minimal requirement) that '...at least one R is different from hydrogen.' The IPEA is of the opinion that a linear polyamine chain with only one further substituent on the terminal amino group still constitutes a linear chain and not a branched chain. Let alone that the polyamine side chains of the partial formula XII and XIII (Claim 25) are only distinguished from D4 by an additional methyl group on the middle nitrogen which despite this minor modification is still deemed to be a linear polyamine chain. It is already now emphasized that in the reg. the Applicant will again be invited to file comparative data.

As concerns the document **WO 02/077007** which is an earlier Application of LEO PHARMA the IPEA assumes that the present application is entitled to the priority date claimed.

For the assessment of the present claims 33-36 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

# CLAIMS

1. A compound according to formula I



wherein the fused rings A, B, C and D are independently saturated or fully or partially unsaturated;

the bond between C-17 and C-20 is depicted with a full and a dotted line to indicate that said bond can be a single or a double bond;

- wherein R1 is hydrogen, halogen, a lipophilic group,  $-(Z)_n-(NR-Z)_p-N(R)_2$  or  $C(O)-(Z)_n-(NR-Z)_p-N(R)_2$ , wherein n is 0 or 1 and p is an integer from 1 and 5; each Z independently represents straight or branched hydrocarbon diradical, optionally substituted with  $C_{1-6}$  alkyl,  $C_{1-6}$  alkenyl,  $C_{1-6}$  alkynyl, hydroxy, alkoxy, amino,  $C_{1-6}$  aminoalkoxy,  $C_{1-6}$  aminoalkyl,  $C_{1-6}$  aminoalkylaminocarbonyl,  $C_{1-6}$  alkyl $C_{3-8}$  cycloalkyl or  $C_{1-6}$  alkylheteroaryl;
- each R independently represents hydrogen or  $C_{1-6}$  alkyl,  $C_{1-6}$  aminoalkyl,  $C_{1-6}$  aminoalkoxy or  $C_{1-6}$  aminoalkylaminocarbonyl, all of which are optionally substituted with alkyl or  $C_{1-6}$  aminoalkyl;
- provided that at least one Z is substituted with  $C_{1-6}$  alkyl,  $C_{1-6}$  alkenyl,  $C_{1-6}$  alkynyl, hydroxy, alkoxy,  $C_{1-6}$  aminoalkoxy,  $C_{1-6}$  aminoalkyl,  $C_{1-6}$  aminoalkylaminocarbonyl,  $C_{1-6}$  alkyl $C_{3-8}$  cycloalkyl or  $C_{1-6}$  alkylheteroaryl, or at least one R is different from hydrogen;
- R2 represents halogen,  $C_{1-4}$  alkyl, optionally substituted with COOH;  $C_{1-4}$  alkoxy, -COOH,  $-(Z)_n-(NR-Z)_p-N(R)_2$  or  $C(O)-(Z)_n-(NR-Z)_p-N(R)_2$ ;
- R3 represents hydrogen halogen or O-R19, wherein R19 represents hydrogen, -SO<sub>3</sub>,  $C_{1-6}$  alkyl,  $C_{1-6}$  acyl or  $-(Z)_n-(NR-Z)_p-N(R)_2$ ;
- each of R4, R7, R8, R11, R12, R13, R16 and R17 independently represent hydrogen, halogen, hydroxy, -OSO<sub>3</sub>, -O-acyl,  $-(Z)_n-(NR-Z)_p-N(R)_2$  or

$C(O)-(Z)_n-(NR-Z)_p-N(R)_2$ ;

R10 represents hydrogen, methyl, halogen, hydroxy,  $-OSO_3$ ,  $-O$ -acyl,  $-(Z)_n-(NR-Z)_p-N(R)_2$  or  $C(O)-(Z)_n-(NR-Z)_p-N(R)_2$ ;

each of R5, R6, R9, R14, R15 and R16 independently represent hydrogen or methyl or are each independently absent when one of the fused rings, A, B, C and D are unsaturated so as to complete the valency of the carbon atom at that site;

provided that at least one, and not more than three of R1, R2, R4, R7, R8, R10, R11, R12, R13, R16 and R17 is  $-(Z)_n-(NR-Z)_p-N(R)_2$  or

$C(O)-(Z)_n-(NR-Z)_p-N(R)_2$ ;

provided that the compound is not

3 $\beta$ -hydroxy-6 $\beta$ -(2-dimethylaminoethyl)amino-5 $\alpha$ -stigmastane,

3 $\beta$ -hydroxy-6 $\beta$ -(2-diethylaminoethyl)amino-5 $\alpha$ -stigmastane,

3 $\beta$ -hydroxy-6 $\beta$ -(3-dimethylaminopropyl)amino-5 $\alpha$ -stigmastane,

3 $\beta$ -hydroxy-6 $\alpha$ -(2-diethylaminoethyl)amino-5 $\alpha$ -stigmastane,

3 $\beta$ -hydroxy-6 $\beta$ -(2-dimethylaminoethyl)amino-5 $\alpha$ -cholestane,

3 $\beta$ -hydroxy-6 $\beta$ -(2-diethylaminoethyl)amino-5 $\alpha$ -cholestane,

3 $\beta$ -hydroxy-6 $\beta$ -(3-dimethylaminopropyl)amino-5 $\alpha$ -cholestane,

3 $\beta$ -hydroxy-6 $\alpha$ -(2-diethylaminoethyl)amino-5 $\alpha$ -cholestane,

20-( $\gamma$ -diethylaminopropyl)-amino-5 $\alpha$ -pregnan-3 $\beta$ -ol,

20-( $\beta$ -diethylaminoethyl)-amino-5 $\alpha$ -pregnan-3 $\beta$ -ol,

20-( $\beta$ -dimethylaminoethyl)-amino-5 $\alpha$ -pregnan-3 $\beta$ -ol,

20-( $\beta$ -dimethylaminoethyl)-aminopregn-5-en-3 $\beta$ -ol,

20-( $\beta$ -diethylaminoethyl)-aminopregn-5-en-3 $\beta$ -ol,

N( $\beta$ -diethylaminoethyl)-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ -trihydroxy-5 $\beta$ -cholan-24-amide,

N( $\beta$ -diethylaminoethyl)-3 $\alpha$ ,12 $\alpha$ -dihydroxy-5 $\beta$ -cholan-24-amide,

N( $\beta$ -diethylaminoethyl)-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ -trihydroxy-5 $\beta$ -cholan-24-amine, or

N( $\beta$ -diethylaminoethyl)-3 $\alpha$ ,12 $\alpha$ -dihydroxy-5 $\beta$ -cholan-24-amine, and

and pharmaceutically acceptable salts or esters thereof.

2. A compound according to claim 1, wherein R2 represents  $-(Z)_n-(NR-Z)_p-N(R)_2$  or  $C(O)-(Z)_n-(NR-Z)_p-N(R)_2$ .

3. A compound according to claim 1, wherein R7, R11 and/or R16 represents  $-(Z)_n-(NR-Z)_p-N(R)_2$  or  $C(O)-(Z)_n-(NR-Z)_p-N(R)_2$ .

4. A compound according to claim 1, wherein R1 represents a lipophilic group.



3  
5  
6. A compound according to claim 1, wherein R1 is selected from the group consisting of straight or branched, saturated or unsaturated C<sub>1-10</sub>alkyl, aryl, C<sub>3-8</sub>cycloalkyl, aralkyl with 1-10 carbon atoms in the alkyl moiety, C<sub>1-10</sub>alkylaryl, C<sub>1-10</sub>alkyl-C<sub>3-8</sub>cycloalkyl, C<sub>1-10</sub>alkoxy and heteroaryl.

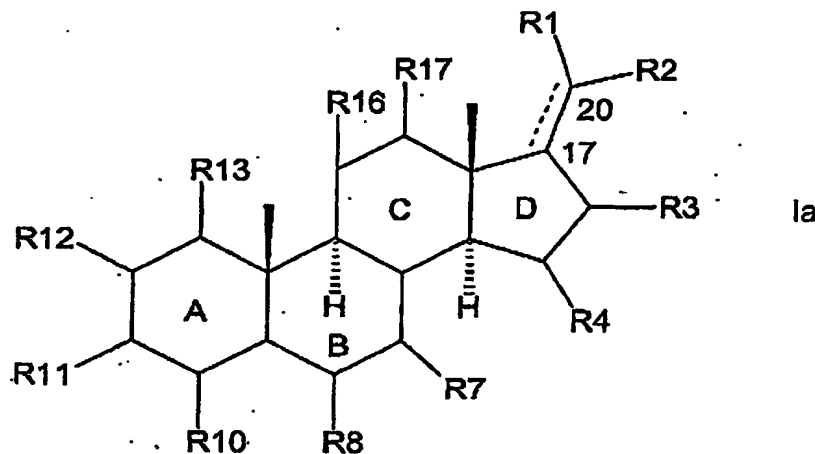
6. A compound according to any of claims 1-5, wherein R19 represents C<sub>1-6</sub>alkyl or C<sub>1-6</sub>acyl.

10 7. A compound according to any of claims 1-6, wherein R7, R11 and/or R16 represents OH

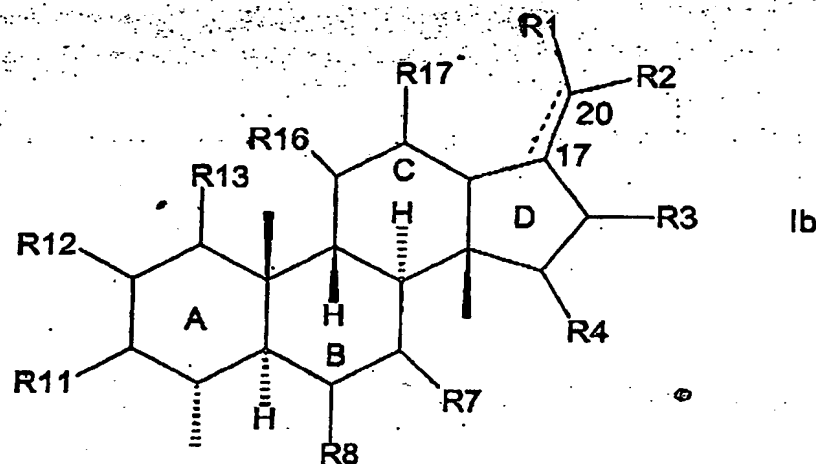
8. A compound according to any of claims 1-5, wherein R11 represents -OSO<sub>3</sub>.

15 9. A compound according to any of claims 1-5, wherein R11 represents -O-acyl.

10. A compound according to claim 1 which has the general formula Ia



20 11. A compound according to claim 1 which has the general formula Ib



12. A compound according to claim 10 or 11, wherein R2 represents  $-(Z)_n-(NR-Z)_p-N(R)_2$  or  $C(O)-(Z)_n-(NR-Z)_p-N(R)_2$ .

13. A compound according to claim 12, wherein R7 and R11 are both hydroxy.

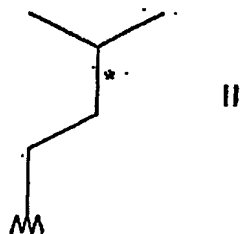
14. A compound according to claim 12, wherein R11 and R16 are both hydroxy.

15. A compound according to claim 12, wherein R3 is  $-OR_{19}$ , wherein R19 is  $C_{1-6}$ alkyl or  $C_{1-6}$ acyl.

16. A compound according to claim 12, wherein R1 is a lipophilic group.

17. A compound according to claim 12, wherein R1 is a straight or branched, saturated or unsaturated  $C_{1-10}$ hydrocarbon.

18. A compound according to claim 12, wherein R1 is a moiety of formula II



wherein the carbon-carbon bond denoted "\*" is a single or double bond.

19. A compound according to claims 10 or 11, wherein R11 represents  $-(Z)_n-(NR-Z)_p-N(R)_2$  or  $C(O)-(Z)_n-(NR-Z)_p-N(R)_2$ .

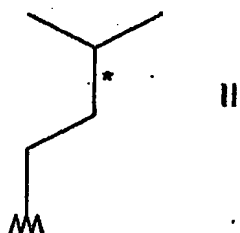
20. A compound according to claim 19, wherein R2 is  $C_{1-4}$ alkyl, optionally substituted with COOH,  $C_{1-4}$ alkoxy or COOH.

21. A compound according to claim 19, wherein R3 is  $O-R_{19}$ , wherein R19 represents  $C_{1-6}$ alkyl or  $C_{1-6}$ acyl.

22. A compound according to claim 19, wherein R1 is a lipophilic group.

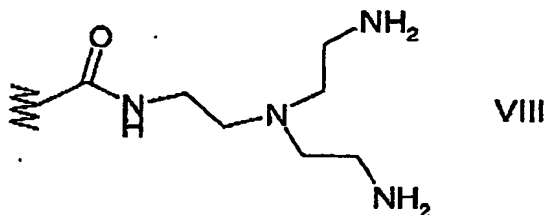
23. A compound according to claim 19, wherein R1 is a straight or branched, saturated or unsaturated  $C_{1-10}$ hydrocarbon.

24. A compound according to claim 19, wherein R1 is a moiety of formula II

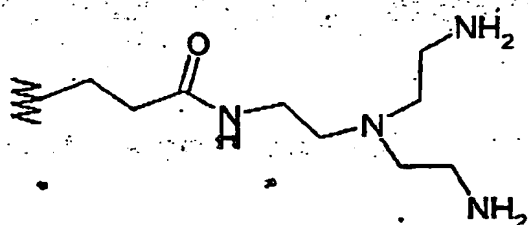


wherein the carbon-carbon bond denoted "\*" is a single or double bond.

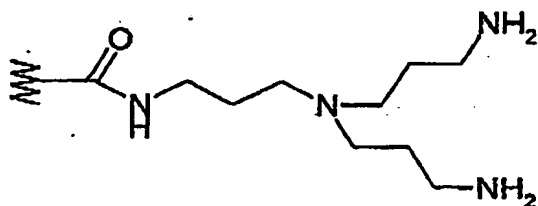
25. A compound according to any one of claims 1, 10 or 11, wherein R2 and/or R11 represents a moiety of the formula VIII, IX, X, XI, XII or XIII



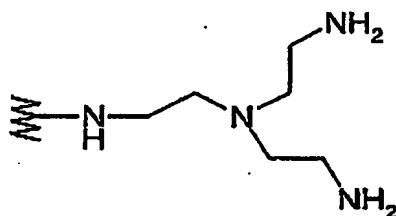
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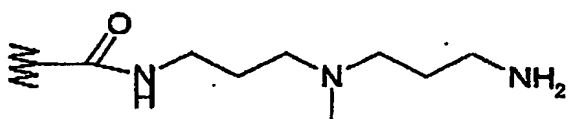
IX



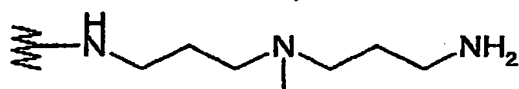
X



XI



XII



XIII

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26. A compound according to claim 1 selected from the list consisting of

- 21-N-{2'-[bis(2'-aminoethyl)amino]ethyl}-17R,20S,24,25-tetrahydrofusid-21-amide,
- 15 21-N-{2'-[bis(2'-aminoethyl)amino]ethyl}-11-desoxy-17R,20S,24,25-tetrahydrofusid-21-amide,
- 21-N-{2'-[bis(2'-aminoethyl)amino]ethyl}-16-desacetoxy-17R,20S,24,25-tetrahydrofusid-21-amide,
- 21-N-{2'-[bis(2'-aminoethyl)amino]ethyl}-13(17)-en-17,20,24,25-tetrahydrofusidan-21-
- 20 carboxamide,

- 21-N-{2'-[bis(2'-aminoethyl)amino]ethyl}-3 $\beta$ -desacetoxy-17R,20S,24,25-tetrahydrofusid-21-amide,  
21-N-{2'-[bis(2'-aminoethyl)amino]ethyl}-9(11)-en-17R,20S,24,25-tetrahydrofusid-21-amide,  
5 24-N-{2'-[bis(2'-aminoethyl)amino]ethyl}-3 $\alpha$ -hydroxy-5 $\beta$ -cholan-24-amide,  
22-N-{2'-[bis(2'-aminoethyl)amino]ethyl}-23,24-bisnor-5-cholenic-22-amide,  
21-N-{2'-[bis(2'-aminoethyl)amino]ethyl}-fusid-21-amide,  
21-N-{3'-[bis(3'-aminopropyl)amino]propyl}-fusid-21-amide,  
21-N-{2'-[bis(2'-aminoethyl)amino]ethyl}-3-OSO<sub>3</sub>-11-desoxy-17,20,24,25-tetrahydro-  
10 fusid-21-amide,  
21-N-{2'-[bis(2'-aminoethyl)amino]ethyl}-11-desoxy-16-desacetoxy-17S,20,24,25-tetrahydrofusid-21-amide,  
21-N-{3'-[bis(3'-aminopropyl)amino]propyl}-17R,20S,24,25-tetrahydrofusid-21-amide,  
22-N-{3'-[bis(3'-aminopropyl)amino]propyl}-23,24-bisnor-5-cholenic-22-amide,  
15 21-N-{3'-[bis(3'-aminopropyl)amino]propyl}-3-OAc-17R,20S,24,25-tetrahydrofusid-21-amide,  
21-N-{3'-[bis(3'-aminopropyl)amino]propyl}-3-OSO<sub>3</sub>-11-desoxy-17,20,24,25-tetrahydrofusid-21-amide,  
21-N-{3'-[bis(3'-aminopropyl)amino]propyl}-11-desoxy-16-desacetoxy-17S,20,24,25-  
20 tetrahydrofusid-21-amide,  
3-N-{2'-[bis(2'-aminoethyl)amino]ethyl}-fusidic acid,  
21-N-{3'-[(3'-aminopropyl)(methyl)amino]propyl}-17R,20S,24,25-tetrahydrofusid-21-amide,  
21-N-{3'-[(3'-aminopropyl)(methyl)amino]propyl}-11-desoxy-17R,20S,24,25-  
25 tetrahydrofusid-21-amide,  
21-N-{3'-[(3'-aminopropyl)(methyl)amino]propyl}-16-desacetoxy-17R,20S,24,25-tetrahydrofusid-21-amide,  
24-N-{3'-[(3'-aminopropyl)(methyl)amino]propyl}-3 $\alpha$ -hydroxy-5 $\beta$ -cholan-24-amide,  
21-N-{3'-[(3'-aminopropyl)(methyl)amino]propyl}-11desoxy-16-desacetoxy-  
30 17R,20S,24,25-tetrahydrofusid-21-amide,  
3-N-{3'-[bis(3'-aminopropyl)amino]propyl}-fusidic acid,  
3-N-{3'-[(3'-aminopropyl)(methyl)amino]propyl}-fusidic acid.

27. A pharmaceutical composition comprising a compound according to any of claims 1-  
35 26, optionally together with a pharmaceutically acceptable excipient or vehicle, and optionally other therapeutically active agents.

28. A composition according to claim 27, wherein said other therapeutically active agent

is selected from the group consisting of penicillins, cephalosporins, tetracyclines, rifamycins, erythromycins, lincomycin, clindamycin, flouroquinolones, corticosteroids, hydrocortosone and triamcinolone.

- 5 29. The use of a compound according to any of claims 1-26 for the manufacture of a medicament for the treatment of prevention of infections.
30. The use according to claim 29, wherein the infection is bacterial
- 10 31. The use according to claim 29, wherein said compound is combined with one or more other therapeutically active ingredients.
- 15 32. The use according to claim 29, wherein said compound is combined with one or more other compounds selected from the group consisting of penicillins, cephalosporins, tetracyclines, rifamycins, erythromycins, lincomycin, clindamycin, flouroquinolones, corticosteroids, hydrocortosone and triamcinolone.
- 20 33. A method of preventing or treating infection, the method comprising administering to a patient in need thereof an effective amount of a compound according to any of claims 1-27.
34. A method according to claim 33, wherein said infection is bacterial.
- 25 35. A method according to claim 33, wherein said compound is administered simultaneously or sequentially with one or more other therapeutically active agents.
- 30 36. A method according to claim 35, wherein said other therapeutically active agent is selected from the list consisting of penicillins, cephalosporins, tetracyclines, rifamycins, erythromycins, lincomycin, clindamycin, flouroquinolones, corticosteroids, hydrocortosone and triamcinolone.